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Notebook labelled on the cover BDIS-USA, John de Kruif.

This is the notebook of John de Kruif, the post-doctoral fellow that performed the experiments together with Leon Terstappen and Ton Logtenberg in the period covering [redacted] until [redacted]. The experiments described in this notebook were performed at Becton Dickinson Immunocytometry Systems (BDIS, San Jose, CA, USA).

General comments:

- In the notebook, a mixture of English and Dutch is used. Dutch words that are used in the notebook are here shown in italics for clarification
- European convention of denoting dates is used (day first, than month and year)
- The dates entered in the notebook do not mention the year. A date is shown on a Fax originating from BDIS is taped in the notebook on page 37 and 38.

The experiments described in this notebook form the basis of the paper by the Kruif et. al, published in the following publication: de Kruif, K., Terstappen, L., Boel, E., and Logtenberg, T. Rapid selection of cell subpopulation-specific human monoclonal antibodies from a synthetic phage antibody library. Proc Natl Acad Sci U S A. 1995 Apr 25;92(9):3938-42.

Description of the most important pages.

- Page 1: the experiments start on [redacted] (di is short for *dinsdag* which means Tuesday in Dutch) with growing up a phage antibody library.
- Page 3: this page dated [redacted] essentially describes the procedure claimed in the patent application.
  - 5.10^6 cells containing B and T lymphocytes (*'cellen: B + T'*) are mixed with 1 ml of the phage (*'faag'*) library (containing approximately 10^{13} phage particles
  - and allowed to incubate overnight at 4^0 C under slow rotation (circular arrow). Although it is not mentioned, the source of the B and T lymphocytes is likely whole blood.

- On the same page, dated [redacted], it is described that the cells are spun down at 1200 rpm, resuspended in 50 ml PBS and spun for 10 minutes at 1200 rpm (to remove unbound phages).
- The cells are subsequently resuspended in 50 µl anti-CD3 antibody conjugated to the fluorochrome FITC (αCD3-FITC and 50 µl of ant-CD20 antibody conjugated to the fluorochrome PE (αCD20-PE).
- The mixture is incubated for 30 minutes (30') on ice ('ijs' in Dutch),
- washed twice as before (*2 x wassen als boven*)
- resuspended (*opnemen*) in 1 ml PBS containing foetal calf serum (PBS-FCS).
- The mixture is subsequently subjected to flow cytometry and cell sorting (FACSSORT in the text).
- Cells from the sorter are collected (*opgevangen*) in 100 µl PBS.
- Collected are: B cells, T cells, eosinophils and 'all cells' (*B,T,Eo's, Alles* in the text). The number of cells sorted is 1,10,100,1000,10.000
- Subsequently it is described that the phages are eluted from the cells by adding 150 µl of 76 mM citric acid (*citroenzuur*), mixed (*mengen*) and incubated for 5 minutes at roomtemperature (*KT*)
- 200 µl of 1 M Tris is added *(gepipetteerd)*
- 1 ml of 2TY medium is added
- 1 ml of bacterial culture in log phase of growth (*logcultuur*) with an optical density of 600 (OD 600 = 0.5-0.8).
- The mixture is incubated for 30 minutes at 370 C (30' 370 C)
- The mixture is spun (round arrow) for 20 minutes at 2000 rpm and the supernatant is almost completely removed (*sup bijna wegzuigen*)
- The mixture is plated out (*uitplaten*).

Note that a section of page 3 has been cut out but that the text continues on the underlying page 5.